

# **Rcbtb2** Cas9-CKO Strategy

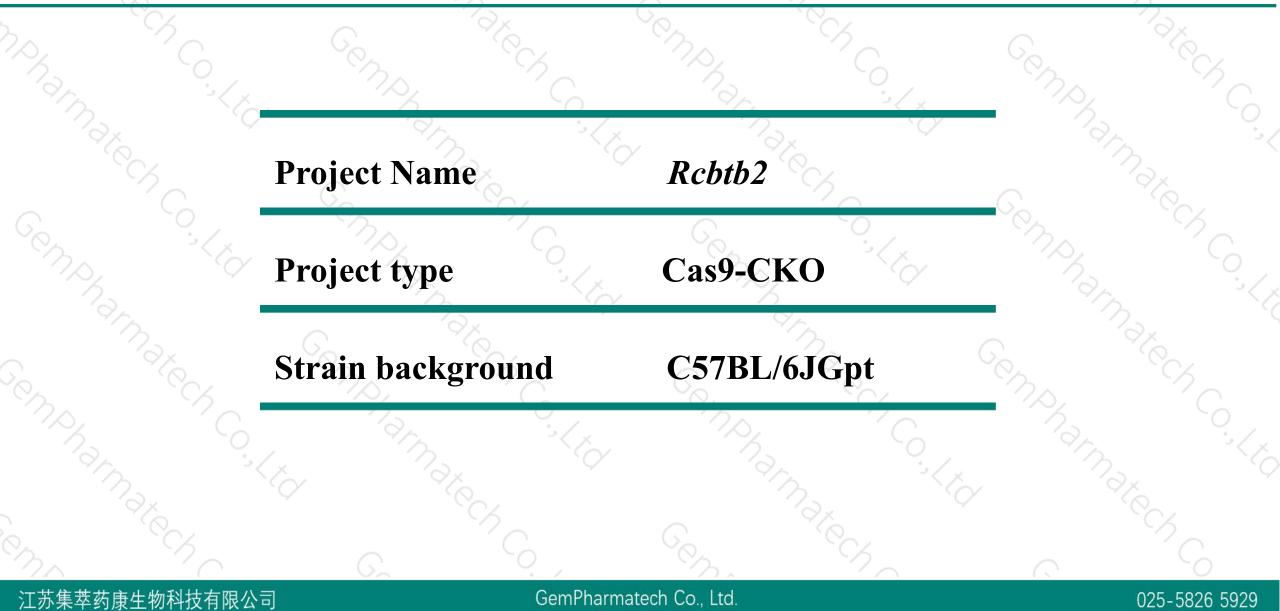
**Designer: Xiaojing Li** 

**Reviewer: JiaYu** 

**Design Date: 2020-8-24** 

# **Project Overview**



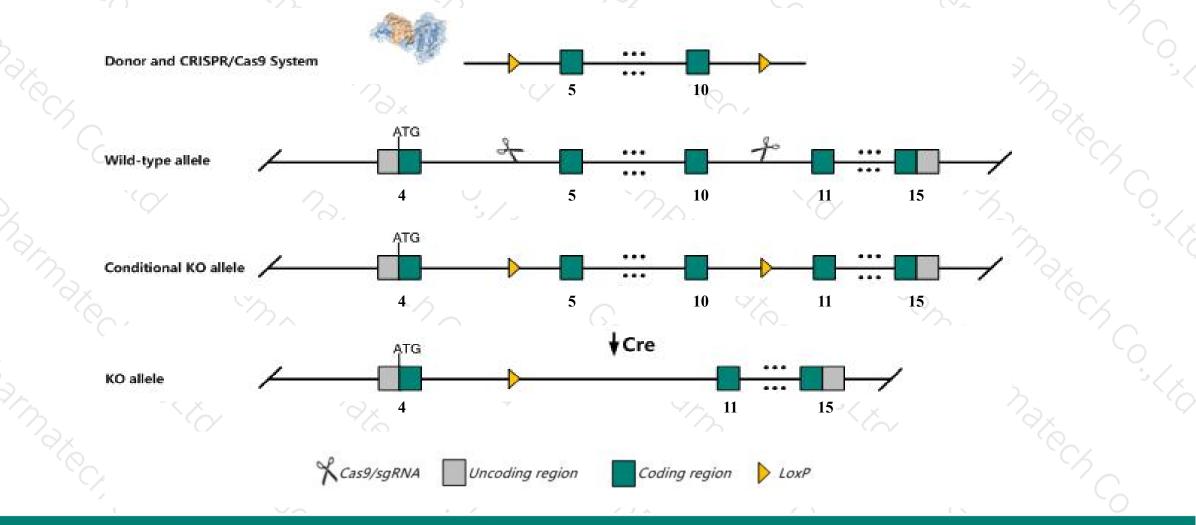


# **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Rcbtb2* gene. The schematic diagram is as follows:



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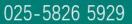
The *Rcbtb2* gene has 26 transcripts. According to the structure of *Rcbtb2* gene, exon5-exon10 of *Rcbtb2-209*(ENSMUST00000164822.7) transcript is recommended as the knockout region. The region contains 884bp coding sequence.
Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rcbtb2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

> The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.



- > The *Rcbtb2* gene is located on the Chr14. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- > This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.



# **Gene information (NCBI)**



### Rcbtb2 regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2 [Mus musculus (house mouse)]

Gene ID: 105670, updated on 13-Mar-2020

#### Summary

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Official Full Nameregulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2 provided byMGIPrimary sourceMGI:MGI:1917200See relatedEnsembl:ENSMUSG0000022106Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Mus; MusAlso known as2610028E02Rik, 2810420M18Rik, Al448345, AW240694, Chc1l, RC/BTB2Orthologshuman all	Official Symbol	Rcbtb2 provided by MGI
See relatedEnsembl:ENSMUSG0000022106Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Mus; MusAlso known as Expression2610028E02Rik, 2810420M18Rik, Al448345, AW240694, Chc1l, RC/BTB2Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissues See more	<b>Official Full Name</b>	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2 provided by MGI
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Organism LineageMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; 2610028E02Rik, 2810420M18Rik, Al448345, AW240694, Chc1l, RC/BTB2 Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissues See more	Gene type	protein coding
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Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as 2610028E02Rik, 2810420M18Rik, Al448345, AW240694, Chc1l, RC/BTB2 Expression Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissues <u>See more</u>	Organism	Mus musculus
Also known as 2610028E02Rik, 2810420M18Rik, Al448345, AW240694, Chc1l, RC/BTB2 Expression Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissues <u>See more</u>	Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
Expression Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissues See more		Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
	Also known as	2610028E02Rik, 2810420M18Rik, AI448345, AW240694, Chc1l, RC/BTB2
Orthologs human all	Expression	Ubiquitous expression in thymus adult (RPKM 22.6), CNS E11.5 (RPKM 17.4) and 28 other tissuesSee more
	Orthologs	human all

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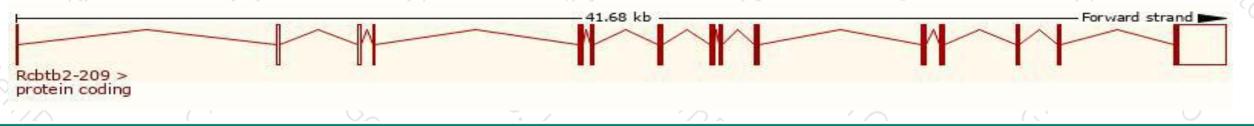
# **Transcript information (Ensembl)**



### The gene has 26 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Rcbtb2-209	ENSMUST00000164822.7	3531	<u>551aa</u>	Protein coding	CCD527266	Q99LJ7	TSL:1 GENCODE basic APPRIS P2		
Rcbtb2-218	ENSMUST00000169479.7	3351	<u>551aa</u>	Protein coding	CCD527266	<u>099LJ7</u>	TSL:1 GENCODE basic APPRIS P2		
Rcbtb2-201	ENSMUST00000022702.12	2971	<u>551aa</u>	Protein coding	CCDS27266	<u>Q99LJ7</u>	TSL:5 GENCODE basic APPRIS P2		
Rcbtb2-202	ENSMUST00000110952.9	2176	<u>551aa</u>	Protein coding	CCD527266	Q99LJ7	TSL:1 GENCODE basic APPRIS P2		
Rcbtb2-219	ENSMUST00000169513.7	3622	<u>527aa</u>	Protein coding	-	<u>099LJ7</u>	TSL:1 GENCODE basic APPRIS ALT2		
Rcbtb2-223	ENSMUST00000170677.7	3573	<u>527aa</u>	Protein coding	-	<u>Q99LJ7</u>	TSL:1 GENCODE basic APPRIS ALT2		
Rcbtb2-226	ENSMUST00000171767.7	2692	<u>517aa</u>	Protein coding	-	E9Q3B1	TSL:1 GENCODE basic		
Rcbtb2-217	ENSMUST00000167401.7	868	<u>72aa</u>	Protein coding	-	E9Q5U1	TSL:5 GENCODE basic		
Rcbtb2-211	ENSMUST00000165429.7	837	<u>157aa</u>	Protein coding	-	<u>E9Q3W8</u>	CDS 3' incomplete TSL:5		
Rcbtb2-204	ENSMUST00000163533.7	762	<u>149aa</u>	Protein coding	-	E9QA48	CDS 3' incomplete TSL:3		
Rcbtb2-212	ENSMUST00000165567.7	749	<u>172aa</u>	Protein coding	-	E9Q7J4	CDS 3' incomplete TSL:5		
Rcbtb2-224	ENSMUST00000171070.7	741	<u>69aa</u>	Protein coding	-	E9PY30	CDS 3' incomplete TSL:5		
Rcbtb2-216	ENSMUST00000167021.7	702	<u>24aa</u>	Protein coding	-	E9Q213	CDS 3' incomplete TSL:5		
Rcbtb2-222	ENSMUST00000170370.7	637	<u>128aa</u>	Protein coding	-	E9QAL7	CDS 3' incomplete TSL:3		
Rcbtb2-221	ENSMUST00000170368.7	559	<u>86aa</u>	Protein coding	-2	E9PZR2	CDS 3' incomplete TSL:5		
Rcbtb2-203	ENSMUST00000163339.7	528	<u>5aa</u>	Protein coding	-		CDS 3' incomplete TSL:3		
Rcbtb2-214	ENSMUST00000165727.7	465	<u>90aa</u>	Protein coding	-	E9QA84	CDS 3' incomplete TSL:3		
Rcbtb2-215	ENSMUST00000166875.1	357	<u>119aa</u>	Protein coding	-	F6QHR6	CDS 5' and 3' incomplete TSL:1		
Rcbtb2-208	ENSMUST00000164298.7	1471	<u>292aa</u>	Nonsense mediated decay	-	F6ZTZ2	CDS 5' incomplete TSL:5		
Rcbtb2-207	ENSMUST00000163797.1	1255	<u>219aa</u>	Nonsense mediated decay	-	F6X714	CDS 5' incomplete TSL:5		
Rcbtb2-225	ENSMUST00000171163.1	2674	No protein	Retained intron	-		TSL:1		
Rcbtb2-220	ENSMUST00000170278.1	1859	No protein	Retained intron	•	150	TSL:1		
Rcbtb2-205	ENSMUST00000163546.7	1462	No protein	Retained intron	-		TSL:1		
Rcbtb2-206	ENSMUST00000163650.7	892	No protein	Retained intron	-	120	TSL:3		
Rcbtb2-210	ENSMUST00000165334.1	649	No protein	Retained intron	-		TSL:3		
Rcbtb2-213	ENSMUST00000165646.7	591	No protein	Retained intron		-	TSL:2		

The strategy is based on the design of *Rcbtb2-209* transcript, the transcription is shown below:



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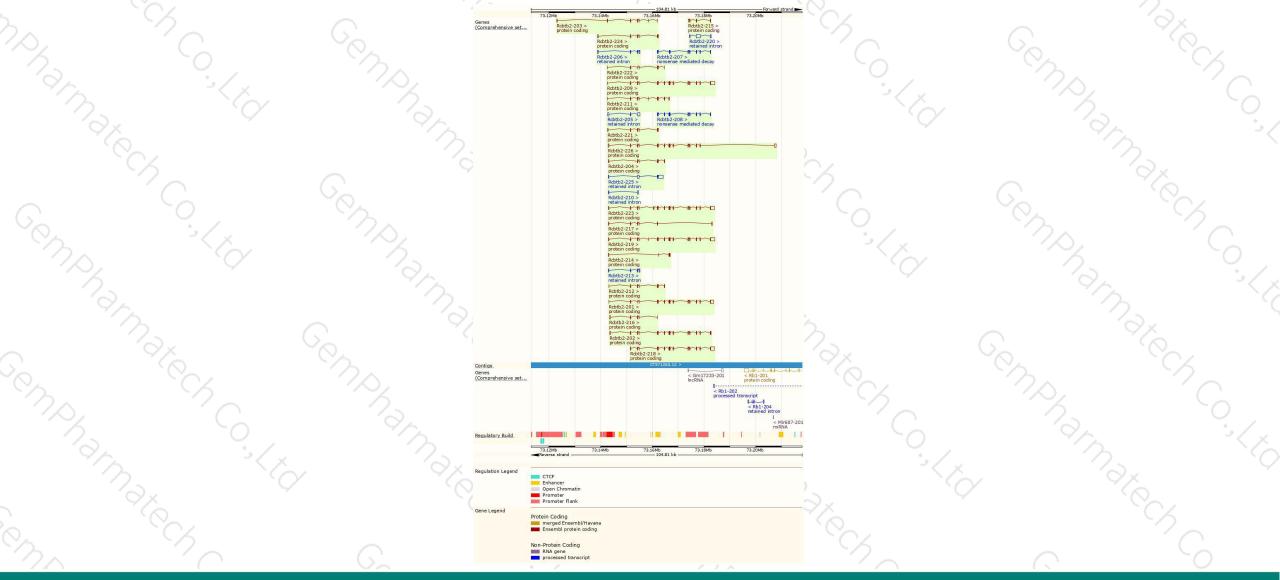
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## **Genomic location distribution**



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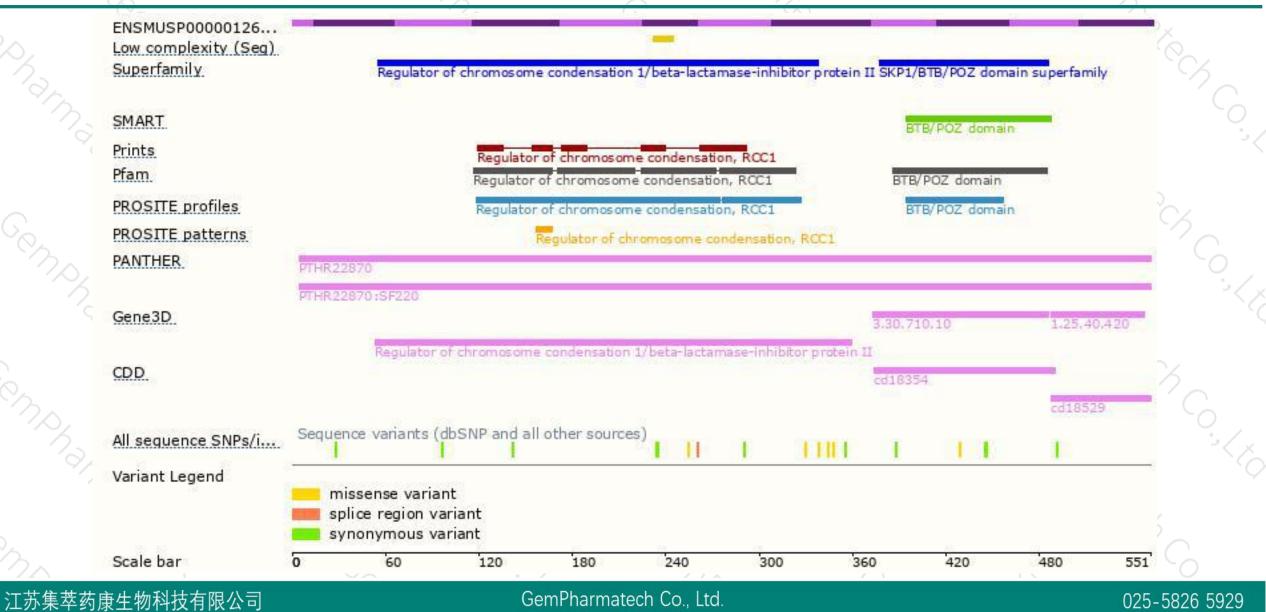


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### **Protein domain**







If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



